JC14 Rec'd PCT/PTO 14 DEC 2001

FORM PTO-1390 (REV 10-95) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. §371	MERCK 2335
	U S. APPLICATION NO. (If known, see 37 CFR §1.5) 10/009635

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP00/05518	15 JUNE 2000	16 JUNE 1999

TITLE OF INVENTION

DEVICE FOR PREPARING SAMPLES

APPLICANT(S) FOR DO/EO/US

EISENBEISS, Friedhelm, et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ■ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. §371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. §371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. §371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. §371(b) and PCT Articles 22 and 39(1).
4. ■ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ■ A copy of the International Application as filed (35 U.S.C. §371(c)(2))
   - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   - b. ■ has been transmitted by the International Bureau.
   - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ■ A translation of the International Application into English (35 U.S.C. §371(c)(2)).
7. ■ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. §371(c)(3))
   - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   - b. ☐ have been transmitted by the International Bureau.
   - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   - d. ■ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. §371(c)(3)).
9. ■ An oath or declaration of the inventor(s) (35 U.S.C. §371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. §371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 C.F.R. §§1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. §§3.28 and 3.31 is included.
13. ■ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☐ Other items or information:

JC13 Rec'd PCT/PTO 14 DEC 2001

U.S. APPLICATION NO. (if known, see 37 CFR §1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
10/009635	PCT/EP00/05518	MERCK 2335

17. ☒ The following fees are submitted: — CALCULATIONS — PTO USE ONLY

BASIC NATIONAL FEE (37 CFR §1.492 (a) (1) - (5)):

Search Report has been prepared by the EPO or JPO..	$890.00
International preliminary examination fee paid to USPTO (37 CFR §1.482).........	$710.00
No international preliminary examination fee paid to USPTO (37 CFR §1.482) but international search fee paid to USPTO (37 CFR §1.445(a)(2))......................	$740.00
Neither international preliminary examination fee (37 CFR §1.482) nor international search fee (37 CFR §1.445(a)(2)) paid to USPTO............................	$1040.00
International preliminary examination fee paid to USPTO (37 CFR §1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)................................	$100.00

				CALCULATIONS	PTO USE ONLY
ENTER APPROPRIATE BASIC FEE AMOUNT =				$890.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 C.F.R. §1.492(e)).					
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	5 - 20 =	0	x $ 18.00	$0.00	
Independent claims	1 - 3 =	0	x $ 84.00	$0.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $ 280.00		
TOTAL OF ABOVE CALCULATIONS =				$890.00	
Reduction of 1/2 for filing by small entity, if applicable. A Verified Small Entity Statement must also be					
SUBTOTAL =				$890.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 C.F.R. §1.492(f)).					
TOTAL NATIONAL FEE =				$890.00	
Fee for recording the enclosed assignment (37 C.F.R. §1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 C.F.R. §§3.28, 3.31). $40.00 per property.					
TOTAL FEES ENCLOSED =				$890.00	
				Amount to be refunded:	
				charged:	

a. ■ A check in the amount of $890.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 13-3402 in the amount of $ ________ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ■ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 13-3402. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 C.F.R. §§1.494 or 1.495 has not been met, a petition to revive (37 C.F.R. §1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO: Customer Number 23,599

23599

PATENT TRADEMARK OFFICE

For [signature] Reg. No. 37,432

SIGNATURE

Anthony J.Zelano

NAME

27,969

REGISTRATION NUMBER

Filed: 14 DECEMBER 2001

AJZ:kmo

APPLICATION DATA SHEET

APPLICATION INFORMATION

Application Type::	REGULAR
Subject Matter::	UTILITY
CD-ROM or CD-R?::	NONE
Title::	DEVICE FOR PREPARING SAMPLES
Attorney Docket Number::	MERCK 2335

INVENTOR INFORMATION

Applicant Authority Type::	INVENTOR
Primary Citizenship Country::	Germany
Status::	FULL CAPACITY
Given Name::	Friedhelm
Family Name::	EISENBEISS
City of Residence::	Weiterstadt
Country of Residence::	Germany
Street of Mailing Address::	Luisenstrasse 28
City of Mailing Address::	Weiterstadt
Country of Mailing Address::	Germany
Postal or Zip Code of Mailing Address::	D-64331

Applicant Authority Type::	INVENTOR
Primary Citizenship Country::	Germany
Status::	FULL CAPACITY
Given Name::	Bernd
Family Name::	STANISLAWSKI
City of Residence::	Frankfurt
Country of Residence::	Germany
Street of Mailing Address::	Ilkenhansstrasse 13
City of Mailing Address::	Frankfurt
Country of Mailing Address::	Germany
Postal or Zip Code of Mailing Address::	D-60433

Applicant Authority Type::	INVENTOR
Primary Citizenship Country::	Germany
Status::	FULL CAPACITY
Given Name::	Thomas
Family Name::	GREVE

City of Residence::	Darmstadt
Country of Residence::	Germany
Street of Mailing Address::	Dieburger Strasse 238
City of Mailing Address::	Darmstadt
Country of Mailing Address::	Germany
Postal or Zip Code of Mailing Address::	D-64287
Applicant Authority Type::	INVENTOR
Primary Citizenship Country::	Germany
Status::	FULL CAPACITY
Given Name::	Renate
Family Name::	BENDER
City of Residence::	Darmstadt
Country of Residence::	Germany
Street of Mailing Address::	Altheimweg 14
City of Mailing Address::	Darmstadt
Country of Mailing Address::	Germany
Postal or Zip Code of Mailing Address::	D-64291
Applicant Authority Type::	INVENTOR
Primary Citizenship Country::	Germany
Status::	FULL CAPACITY
Given Name::	Roland
Family Name::	HERGEN-RODER
City of Residence::	Dortmund
Country of Residence::	Germany
Street of Mailing Address::	Immermannstrasse 35
City of Mailing Address::	Dortmund
Country of Mailing Address::	Germany
Postal or Zip Code of Mailing Address::	D-44147
Applicant Authority Type::	INVENTOR
Primary Citizenship Country::	Germany
Status::	FULL CAPACITY
Given Name::	Gunther
Family Name::	WEBER
City of Residence::	Dortmund
Country of Residence::	Germany
Street of Mailing Address::	Justusweg 2
City of Mailing Address::	Dortmund

Country of Mailing Address::	Germany
Postal or Zip Code of Mailing Address::	D-44149
Applicant Authority Type::	INVENTOR
Primary Citizenship Country::	Germany
Status::	FULL CAPACITY
Given Name::	Benedikt
Family Name::	GRASS
City of Residence::	Werl
Country of Residence::	Germany
Street of Mailing Address::	Schesien Strasse 30
City of Mailing Address::	Werl
Country of Mailing Address::	Germany
Postal or Zip Code of Mailing Address::	D-59457
Applicant Authority Type::	INVENTOR
Primary Citizenship Country::	Germany
Status::	FULL CAPACITY
Given Name::	Andreas
Family Name::	NEYER
City of Residence::	Iserlohn
Country of Residence::	Germany
Street of Mailing Address::	Langerfelderstrasse 69a
City of Mailing Address::	Iserlohn
Country of Mailing Address::	Germany
Postal or Zip Code of Mailing Address::	D-56838
Applicant Authority Type::	INVENTOR
Primary Citizenship Country::	Germany
Status::	FULL CAPACITY
Given Name::	Matthias
Family Name::	JOHNCK
City of Residence::	Munster
Country of Residence::	Germany
Street of Mailing Address::	Dulmener Strasse 27a
City of Mailing Address::	Munster
Country of Mailing Address::	Germany
Postal or Zip Code of Mailing Address::	D-48163
Applicant Authority Type::	INVENTOR
Primary Citizenship Country::	Germany

Status::	FULL CAPACITY
Given Name::	Dirk
Family Name::	SIEPE
City of Residence::	Dortmund
Country of Residence::	Germany
Street of Mailing Address::	Vinckeplatz 7
City of Mailing Address::	Dortmund
Country of Mailing Address::	Germany
Postal or Zip Code of Mailing Address::	D-44139

Applicant Authority Type::	INVENTOR
Primary Citizenship Country::	SLOVAKIA
Status::	FULL CAPACITY
Given Name::	Dusan
Family Name::	KANIANSKY
City of Residence::	Bratislava
Country of Residence::	Slovakia
Street of Mailing Address::	Sevcenkova 28
City of Mailing Address::	Bratislava
Country of Mailing Address::	Slovakia
Postal or Zip Code of Mailing Address::	851 01

DOMESTIC PRIORITY INFORMATION

Application::	Continuity Type::	Parent Application::	Parent Filing Date::
This Application	National Stage of	PCT/EP00/05518	06/15/00

FOREIGN PRIORITY INFORMATION

Application Number::	Country::	Filing Date::	Priority Claimed::
199 27 533.5	Germany	06/16/99	YES
199 27 534.3	Germany	06/16/99	YES
199 27 535.1	Germany	06/16/99	YES
PCT/EP00/05204	EP	06/06/00	YES
PCT/EP00/05205	EP	06/06/00	YES
PCT/EP00/05206	EP	06/06/00	YES

ASSIGNMENT INFORMATION

Assignee Name::	Merck GmbH
Street of Mailing Address::	Frankfurter Strasse 250
City of Mailing Address::	Darmstadt
Country of Mailing Address::	GERMANY

Postal or Zip Code of Mailing Address:: 64293

10009535.121401

10/009655
14 DEC 2001

IN THE UNITED STATES DESIGNATED/ELECTED OFFICE

International Application No.	:	PCT/EP00/05518
International Filing Date	:	15 JUNE 2000
Priority Date(s) Claimed	:	16 JUNE 1999
Applicant(s) (DO/EO/US)	:	EISENBEISS, Friedhelm, et al.

Title: DEVICE FOR PREPARING SAMPLES

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

SIR:

Prior to calculating the national fee, and prior to examination in the National Phase of the above-identified International application, please amend as follows:

IN THE CLAIMS:

3. (Amended) Analytical unit according to Claim 1, characterized in that the fluidic connections are peristaltic pumps, syringes or syringe pumps.

4. (Amended) Use of the analytical unit according to Claim 1 for isotachophoretic separation of a sample.

5. (Amended) Use of the analytical unit according to Claim 1 for depleting matrix components from a primary sample, for extracting analytes from a primary sample, for separating off analytes from the primary sample or for enriching of analytes in minor amounts.

REMARKS

The purpose of this Preliminary Amendment is to eliminate multiple dependent claims in order to avoid the additional fee. Applicants reserve the right to reintroduce claims to canceled combined subject matter.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached pages are captioned "**Version With Markings to Show Changes Made**".

Respectfully submitted,

For Reg. No. 37,432

Anthony J. Zelano, Reg. No. 27,969
Attorney for Applicants
MILLEN, WHITE, ZELANO & BRANIGAN, P.C.
Arlington Courthouse Plaza 1
2200 Clarendon Boulevard, Suite 1400
Arlington, VA 22201
Direct Dial: 703-812-5311
Facsimile: 703-243-6410
Email: zelano@mwzb.com

AJZ:kmo

FILED: 14 DECEMBER 2001

VERSION WITH MARKINGS TO SHOW CHANGES MADE:

Claims 3 - 5 have been amended as follows:

3. (Amended) Analytical unit according to Claim 1~~ or 2~~, characterized in that the fluidic connections are peristaltic pumps, syringes or syringe pumps.

4. (Amended) Use of the analytical unit according to ~~one of~~ Claim~~s~~ 1~~ to 3~~ for isotachophoretic separation of a sample.

5. (Amended) Use of the analytical unit according to ~~one of~~ Claim~~s~~ 1~~ to 3~~ for depleting matrix components from a primary sample, for extracting analytes from a primary sample, for separating off analytes from the primary sample or for enriching of analytes in minor amounts.

10/009635
JC13 Rec'd PCT/PTO 14 DEC 2001

10/prts

Sample preparation apparatus

The invention relates to a miniaturized apparatus for preparing sample material containing analytes in predominantly aqueous solution.

Especially in the analysis and extraction of complex materials, for example cells, blood or food, before the actual analysis or isolation of certain components, complex purification steps have to be carried out in order to free the analytes from the complex and frequently interfering matrix. Further analysis or purification is only possible by this prepurification.

Known sample preparation methods are, for example, precipitations or extractions. However, even after such pretreatments, many samples still remain too complex. A further possibility for sample preparation is chromatographic or electrophoretic methods. However, in order to obtain samples which are sufficiently purified by these methods, much more apparatus is frequently required.

For analytical applications, recently miniaturized microstructured analytical systems having planar channel systems (microchips) have been developed. Miniaturized analytical systems offer the advantage of carrying out generally electrophoretic separations without great expenditure and consumption of reagents. An example of these is the bioanalyser from Agilent Technologies, Germany.

However, these systems are designed exclusively for analytical purposes. However, a precondition for effective and sensitive analysis is frequently effective sample preparation which these systems cannot provide. Only a small part of the sample volume ultimately passes into the separation channel. As a

result it is difficult to introduce a volume element which has a statistically representative composition of the original sample. After analysis has been carried out, the microchip is discarded. The analytes remain on the microchip.

Therefore, analytical systems of this type are not suitable for sample preparation. This results therefore in the object of utilizing the advantageous design of miniaturized analytical systems and developing systems which are suitable in particular for sample preparation. For this purpose the analytical units must comply with the following requirements:

- In contrast to purely analytical systems, in the case of analytical units for sample preparation it is of importance that large sample volumes can be processed.
- If the analytes after purification are to be submitted, for example, to further analyses (for example mass spectrometry) or derivatizations, they must be able to be taken out of the analytical unit for sample preparation.
- Very small amounts of analyte must be able to be isolated from a large amount of sample matrix.
- After purification the analyte should be present in as concentrated a form as possible.

It has been found that the abovementioned requirements for sample preparation are met by a miniaturized analytical unit consisting of a microstructured planar channel system made of plastic as continuous-flow unit, an adaptor chamber for reversibly receiving the continuous-flow unit, a fluidics supply, a power supply and detectors. The inventive analytical unit in particular has an apparatus for precise delivery of large sample volumes above 0.1 µl and preferably an apparatus for discharging sample volumes. Separation is preferably performed isotachophoretically.

The present invention therefore relates to an analytical unit for sample preparation at least comprising a continuous-flow unit made of plastic having a microstructured channel system, an adaptor chamber for reversibly receiving the continuous-flow unit, a fluidics supply, a power supply and at least one detector, characterized in that to receive the sample a channel section is provided at the ends of which in each case are situated fluidic connections.

In a preferred embodiment, the inventive analytical unit has an apparatus for discharging sample volumes, which apparatus essentially consists of a channel system having at least one Y branch, at least three transport electrodes and at least one detection apparatus upstream of the said branching point of the channel system and having an electrical switching apparatus.

In a preferred embodiment, the fluidic connections used are peristaltic pumps, syringes or syringe pumps.

The present invention also relates to the use of the inventive analytical unit for isotachophoretic separation of a sample.

The present invention additionally relates to the use of the inventive analytical unit for depleting matrix components from a primary sample, for extracting analytes from a primary sample, for separating off analytes from the primary sample or for enriching analytes in minor amounts.

Figure 1 shows a possible arrangement of the channel system for the inventive apparatus for sample delivery.

Figure 2 shows a possible procedure in the charging of a miniaturized analytical unit.

Figure 3 shows diagrammatically the channel system of a continuous-flow unit having an inventive apparatus for sample delivery and various possibilities for sample removal.

Figure 4 illustrates the discharge of a substance using the inventive discharge apparatus.

Figure 5 shows a suitable apparatus for the input and/or output of optical power into or out of defined regions of microstructured planar fluidic systems.

Figure 6 shows diagrammatically the connection of fluidics and electrics to a continuous-flow unit.

The explanations to Figures 7 to 10 are found in Examples 3 and 4.

The present invention relates to an analytical unit using which more than 0.1 µl of liquid sample material can be quantitatively removed, purified in a controlled manner using electrophoretic processes and optionally can be analysed or fed to other systems. The total concentration of dissolved ionic components in this case can be in a range from 1 µM to 10 mM.

The inventive analytical unit for sample preparation consists at least of the following constituents:

- a continuous-flow unit made of plastic having a microstructured channel system
- an adaptor chamber for reversibly receiving the continuous-flow unit
- a fluidics supply
- a power supply
- at least one detector

The continuous-flow unit is, in addition, structured so that an apparatus for delivering sample volumes between 0.1 and, depending on the system, typically 20 µl with a deviation of less than 5% is present. If the

remaining instrument parameters of the analytical unit, for example the power supply, which are of importance for effective separation and analysis, are adapted appropriately, even greater sample volumes can be delivered. The sample volume is defined only by the volume of the channel section which is limited by the fluidics connections. Preferably, in addition, an apparatus for discharging sample volumes is integrated.

The structure of the individual parts of the inventive analytical units is described below.

Continuous-flow unit:

The core of microfluidic or microstructured systems is generally a continuous-flow unit which has at least the channel system and optionally recesses for integrating peripheral devices, and peripheral devices, such as detectors, fluidics connections, reservoirs, reaction chambers, pumps, control apparatuses etc. which can be integrated into the continuous-flow unit or connected thereto. Suitable continuous-flow units for an analytical unit are according to the invention systems in which, by joining together at least two components, for example substrate and cover, microchannel structures are produced which can be sealed liquid-tightly and/or gas-tightly.

The channel system of the continuous-flow unit typically has two or more channel segments for receiving separation buffers. These channel segments are each provided with fluidics connections for introducing and removing the buffers. If the channel segments additionally serve as separation channel, the fluidics connections can also be utilized for removing analytes or matrix components.

In addition, the channel system comprises at least one channel segment for receiving the sample with fluidics

connections for introducing the sample and for removing the purified sample or the matrix.

Preferably, using the inventive analytical unit, the samples are fractionated isotachophoretically, since this gives the possibility of enriching very small amounts of analytes from large sample volumes and separating them. For this, the continuous-flow unit must permit the sample volume to be introduced at the start of the isotachophoretic separation directly between two zones of aqueous buffers, in which case one buffer, the leading buffer, has ions of higher electrophoretic mobility than the sample components to be analysed and the other buffer, the terminating buffer, has ions of lower electrophoretic mobility.

The components of the continuous-flow unit of the analytical unit preferably consist of commercially available thermoplastics, such as PMMA (polymethyl methyacrylate), PC (polycarbonate), polystyrene or PMP (polymethylpentene), cycloolefinic copolymers or thermosetting plastics, for example epoxy resins. Preferably, all components of the system consist of the same material.

The components can be fabricated by methods known to those skilled in the art. Components which comprise microstructures can be produced, for example, by established methods, such as hot-embossing, injection moulding or reaction casting. Particularly preferably, components are used which can be duplicated by known methods of mass production. Microstructured components can have channel structures with cross-sectional areas between 10 and 250 000 μM^2.

For the integration of electrodes into the continuous-flow unit, the electrodes are preferably mounted on a component of the system, the cover. For this purpose they must have an adequate adhesion strength on the

plastic component. This is of importance both for joining together the individual components and for the later use of the entire apparatus. If adhesives, for example, are used during the joining of the components, the adhesive must not detach the electrode from the plastic surface. In addition, the electrodes should consist of chemically inert materials, for example noble metals (platinum, gold).

Plastic surfaces are typically metallized by electrochemical deposition of metals from metal salt solutions. For this it is generally customary, in any multistep process, first to pretreat the plastic surface chemically or mechanically, to apply a discontinuous primer and then to carry out the electrochemical deposition. Descriptions of these metallization techniques may be found, for example, in US 4,590,115, EP 0 414 097, EP 0 417 037 and in Wolf and Gieseke (G.D. Wolf, H. Gieseke, "Neues Verfahren zur ganzflächigen und partiellen Metallisierung von Kunststoffen" [Novel Process for complete and Partial Metallization of Plastics], Galvanotechnik 84, 2218-2226, 1993). All of the wet-chemical processes share the fact that relatively complex pretreatment processes are necessary to achieve sufficient adhesion strengths.

DE 196 02 659 describes the application of copper with strong adhesive strength to multiphase polymer blends using vaporization or sputtering. The reason mentioned for the good adhesion is the composition of the polymer blends. According to this the blends must comprise polyarylene sulphides, polyimides or an aromatic polyester.

The effect of plasma pretreatment to achieve improved adhesion properties of metals on plastic surfaces is summarized by Friedrich (J. Friedrich, "Plasmabehandlung von Polymeren" [Plasma Treatment of Polymers], kelben & dichten 41, 28-33, 1997) for the

example of various commercially available thermoplastics.

Particularly preferably, the electrode structures are produced on the plastic components using a two-layer method. For this purpose, first an adhesion-promoting layer of chromium oxide is produced. In contrast to noble metals, chromium oxide displays outstanding adhesion properties on plastic surfaces. In addition, chromium oxide, in contrast to elemental chromium and other transition metals, is considerably more resistant to redox processes. The noble metal, for example platinum or gold, or alloys of these metals, is then applied to the adhesion layer of chromium oxide.

Chromium oxide and the noble metal layer to be deposited thereon is preferably selectively applied to plastic substrates in the lift-off process or by means of the shadow-mask method or the structuring of metal layers initially applied on whole surfaces. These operations are standard processes in microstructuring. Below, the working steps necessary for the two-layer method are briefly described for the said processes.

Lift-off process: the plastic component to be metallized selectively is coated with a photoresist. This photoresist must not etch the plastic part to be metallized, or etch it only slightly. For PMMA, for example, a photoresist from Allresist, Berlin (AR 5300/8) has proved to be suitable. After exposure and development of the structures to be metallized, the metal layers are applied in a sputtering unit. The chromium oxide layer is applied during the sputtering process by introducing oxygen into the sputtering system argon plasma typically used. The sputtering target used is a conventional chromium target. Typical chromium oxide layer thicknesses are 10-50 nm. Alternatively, a chromium oxide target can be used directly. Platinum or its alloys or gold is then

sputtered directly under standard conditions, i.e. in the argon plasma. It has also proved to be advantageous for the adhesion strength of the chromium oxide layer to backsputter the plastic in an oxygen/argon (approximately 5% by volume/95% by volume) plasma before sputtering the chromium oxide. In the actual lift-off process, the photoresist still present, and together with this the metal layer situated on the resist, are detached from the plastic component in a developer from Allresist (AR 300-26).

Shadow mask technique: the plastic part to be metallized selectively is covered with what is termed a shadow mask. This has openings at the areas to be metallized. The metal layers are sputtered through these similarly to the lift-off process.

Structurizing planar metal layers: a metal layer is firstly applied, similarly to the above described sputtering process, over the whole surface of a plastic part to be metallized selectively. This metal layer is structured in subsequent process steps, either by selective erosion using, for example, laser ablation (gold and platinum) or, for example, by selective wet-chemical etching. For structuring using wet-chemical etching, a photoresist (Hoechst AG, Germany; AZ 5214) is first applied to the metal layer, exposed and developed. Gold is then removed in the exposed areas in cyanide solution. The non-electrically-conducting chromium oxide layer remains behind. The remaining photoresist is then removed with a developer (for example AR 300-26, Allresist, Berlin).

The adhesion strength of electrodes produced with chromium and also chromium oxide as adhesion layer using the sputtering method has been tested using tear-off tests. The adhesion strength of the chromium oxide layers is significantly greater. The metal layers that were produced using chromium oxide as adhesion layer

are also markedly more resistant in ultrasonic treatment in alkaline solution compared with metal layers which were produced using chromium as adhesion layer.

After production and preparation of individual components, these are joined together. Preferably, one component, the substrate, is microstructured and provided with rear-side bore holes or recesses for filling the channels and/or contacting the electrodes. In addition, the use of a so-called sealing lip, that is to say an elevation on the substrates which completely encloses the channel structures with a height between typically 0.5 and 5 µm, has proved to be highly advantageous with respect to the adhesion process. The other component, the cover, serves for covering and, for example, is provided with the electrodes in the case of electrophoretic analytical units. In this case the cover according to the invention is termed electrode cover. For certain applications, the continuous-flow units can require a functionalization of the component deviating from this preferred arrangement. In this case, for example, more than two components, for example two covers and one substrate etc., can be joined together in order to generate channel structures which lie one above the other, or other functionalities, such as detection systems, reaction chambers etc., can be integrated into the components. According to the invention, all parts of the continuous-flow unit which are joined together using a bonding process are termed components. They can be microstructured, provided with electrodes or have other functionalities. A subdivision of the components into substrates and covers or electrode covers, if the respective component is provided with electrodes, only serves for the more detailed description of the embodiment of the specific components and does not represent any restriction with respect to other

properties of the components, such as microstructuring etc., or their combinations with one another.

The components are preferably joined together with high precision using an adhesive method. The adhesive must not run into the channels and coat their surfaces, since this can alter the surface properties of the channels. It has been found that this leads, for example, to increased adhesion of analytes, for example proteins, in the channel regions which are wetted with adhesive. This in turn affects the separation quality. Similarly, sticking of adhesive to the electrodes impairs their functionality.

It is also of great importance that the volume of the channels is not changed, as would occur, for example, by the uncontrolled ingress of adhesive. According to the invention the channel, to improve the sensitivity of detection, is preferably constricted in the vicinity of the detection electrodes. As a result it is important precisely in these areas that no adhesive passes into the channel.

To join the components together, according to the invention an adhesive is preferably applied firstly to the microstructured component at the points at which no structuring is present. The layer thickness is between 0.5 and 10 µm, preferably between 3 and 8 µm. Typically, it is applied using a flat roller application known from printing technology.

In a preferred embodiment, a thin adhesive film is applied here via a structured metallic screen roll which takes up a defined volume of adhesive, to a second unstructured roll which is coated with a polymer. From this, in turn, application is performed directly onto the structured substrate in such a manner so as preferably to give an adhesive thickness between 3 and 8 µm on the unstructured surface of the

substrate. Depending on the plastic (substrate material) used, the transfer between the plastic roll and the substrate is influenced by any viscosity increase in the adhesive (prepolymerization). An important advantage of this process is that the substrate need not be positioned relative to the roll bearing the adhesive and nevertheless adhesive is only applied to the unstructured regions of the substrate. If too much adhesive is applied, when the cover and substrate are pressed together, adhesive will flow into the channel. If in parts insufficient adhesive has been applied, leaks in the channel structure result. This bonding method requires a flatness of the components of preferably less than approximately 5 μm/cm of component length.

The adhesive used must not etch the surface of the components or etch it only slightly, in order that the electrodes during the adhesion process are not detached or interrupted by the adhesive. Preferably, therefore, the adhesive used is the product NOA 72, thiolacrylate from Norland, New Brunswick NJ, USA. This glue is cured photochemically. However, other types of glues can also be used for the process, for example thermally curing glues which comply with the above conditions.

After the adhesive has been applied, the second component having the thin-layer electrodes is positioned appropriately to the substrate for example on an exposure machine and pressed on. For this purpose, preferably the substrate together with the applied adhesive is fixed in the exposure machine in the position otherwise provided for silicon wafers. Preference is given to the use of thick glass plates as pressing surface, since in this manner the positioning and photochemical curing of the glue can be carried out by illumination with an Hg lamp (emission wavelength 366 nm). The electrode cover is fixed in the position provided for the exposure mask by holding it with a

vacuum apparatus milled in a glass plate. Since both the electrode cover and the glass plate used for holding the cover are transparent, the cover can be aligned with respect to the substrate through this arrangement. If the cover extends beyond the substrate, this cover can also be held mechanically.

For the adhesion process, typically in addition to optomechanical alignment with the assistance of optical alignment marks, the cover can also be positioned on the substrate passively mechanically using a push-in apparatus, optomechanically without special alignment marks, or electromechanically using electrical marks (contacts).

It has been found that the preferred optical metallic alignment marks on the cover can be applied together with the electrodes in the same process step, that is to say preferably can be sputtered, that is to say no additional expenditure is necessary. The corresponding counterstructures on the substrate also require no additional processing, since these are introduced into the substrate together with the channel structures in a moulding step. For the optomechanical alignment, at least one component must consist of a transparent plastic. Using the inventively applied alignment marks, the two components are positioned with respect to one another at an accuracy of at least ± 10 µm, typically even ± 2 µm (for example of theoretical to actual position of the detector electrode) and pressed together. The high position accuracy supports the achievement of reproducible separation results and analytical results. The adhesive is then polymerized by a UV lamp. After turning off the vacuum for holding the cover or releasing the mechanical fixing the continuous-flow unit is removed from the exposure machine.

In another preferred embodiment, a component is provided with adhesive by means of a process known in printing (pad printing). The component provided with the electrodes is wetted with the glue for this purpose on the regions which, when the two components are combined, must not lie over a channel or be electrically contacted. Microstructured components are wetted such that no adhesive passes into the channel structure or other recesses. Pad printing is a structured glue application. Adhesive is stored in a negative mould of the substrate. Via a typically silicone pad, this adhesive is taken up in a structured manner and applied, for example, onto the cover in such a manner that the regions which later form a wall of a fluidic channel are not wetted with the adhesive. The component having the channel structures is then, as described above, positioned in a suitable manner to its counterpiece and pressed on. Curing is performed as described above.

A structured glue application using spray methods (for example microdrop methods) or using screen-printing methods is possible, provided that the lateral resolution of the glue discharge is sufficient.

Pressing on the second component or pressing together the components, for the purposes of the invention, means that the components are suitably brought into contact with one another. In order to achieve, after curing, a permanent bonding of the components, it is generally not necessary to exert a large force, that is to say to press the components together very firmly.

If the curing process of the glue is carried out outside the alignment apparatus used for positioning the cover and substrate, the metallized cover and the substrate, after they have been aligned with one another, can be initially attached by laser welding. After this the composite is removed from the alignment

apparatus and the adhesive used is cured in a separate exposure apparatus or an oven. This procedure means a process acceleration and simplification, since the curing need no longer be performed in the alignment apparatus.

Since the thermoplastic materials preferably used are very largely transparent to laser light in the visible and near-infrared wavelength region, laser welding in this wavelength range requires an absorber layer for absorbing the optical power at the interface between cover and substrate. This absorber layer is applied simultaneously with the application of the power electrodes or detector electrodes. For example, the electrode cover, during sputtering of the electrodes with noble metal, can additionally be sputtered at further points with a noble metal layer as absorber layer.

Welding an electrode cover which is provided with 200 nm thick platinum electrodes and also comprises additional platinum surfaces for absorbing the laser power to a substrate (base material PMMA) is performed using diode laser radiation (mixture of wavelengths of 808, 940 and 980 nm) at a power of 40 watts and a focus diameter of 1.6 mm. The platinum layer is destroyed during welding.

Alternatively, it is also possible to use a substrate or cover filled, for example, with carbon black particles, as absorber. This last-mentioned procedure, however, has the disadvantage that then at least one channel wall consists of another material. The possibilities for input into the channel or output from the channel of optical power for optical detection purposes are also restricted thereby.

The contacting of the transport electrodes and detection electrodes and of the automatic control and

switching of the electric current are made by methods known to those skilled in the art.

The microstructuring of the components, that is to say the design of the channel system, can be adapted to the respective application. The detection electrodes can be positioned at any desired position in the channel system. However, particularly preferably they are positioned at constriction points at which the channel system has a smaller diameter. As a result, particularly good analytical resolution is achieved during detection.

Preferably a continuous-flow unit has a plurality of segments of separation channels in a series arrangement or in a branched arrangement. The segments have a relatively high cross-sectional area of typically 0.01 to 1.0 mm^2. Between the segments are constriction points having cross-sectional areas < 0.01 mm^2, which can optionally be provided with a detector during fabrication. In this manner it is possible to fabricate continuous-flow units having differing volume ratios of separation channel to primary sample (corresponds to channel segment to sample delivery) by varying the position of the detector using one construction pattern for the capillary structure. The range of the volumetric ratio of separation channel/primary sample extends typically from 2/1 to 30/1. For small volumetric ratios, separation with low resolution in a short time is possible, in the case of high volumetric ratios, separation with high resolution for a longer separation time can be carried out.

Sample delivery apparatus:

This apparatus is produced by the design of the channel system of the continuous-flow unit and integration of fluidics connections.

In contrast to other delivery methods, in the inventive apparatus the channel system is open at two positions during sample delivery. One opening serves for introducing the liquid, that is to say for example the sample solution, the other opening enables the exit of the liquid or air previously present in the system. The principle of the inventive delivery apparatus is therefore the displacement by the sample solution of a volume of liquid or gas present in a defined channel section.

By suitable choice of the inlet and outlet openings, only the liquid in the intermediate channel section is displaced, or the intermediate channel section is filled. The liquid in any adjacent side channels present is not replaced, since there are no open inlet or outlet openings in the side channels and thus the liquid in these regions is moved neither by pressure nor by suction. Losses or dilutions by liquid streams at the contact areas to side channels are low in relation to the entire sample volume, which is typically in the µl region. In the case of a suitable constant metering rate, the sample delivery can be made very reproducibly. This is a great advantage compared with methods in which very small sample volumes of a few nanolitres are delivered. In principle, an inventive delivery apparatus is also suitable for delivery volumes of less than 50 nl. However, compromises are then necessary with respect to precision and accuracy.

The sample liquid can be transported via fluidic connections, that is to say closely connected pumps, syringes, micromixers, electroosmosis or hydrostatic pressure, preferably via pumps and valves.

These apparatuses can be mounted preferably externally, as close as possible to the continuous-flow unit.

The outflowing liquid need not be additionally pumped off. It is sufficiently effectively displaced by the pressure of the injected replacement liquid.

This type of filling avoids the disadvantages of electroosmotic injection, that is to say filling is largely independent of sample composition, pH and material of the continuous-flow unit. The valves or tightly sealing pumps present prevent any interfering liquid motion, for example due to hydrostatic pressure differences or electroosmosis.

Viscosity and ionic strength of the sample solution or of the solution to be displaced, that is to say, for example, a transport buffer, have only a small effect on the metering or filling rate. It is possible to charge suspensions, emulsions, particle-containing liquids and cell-containing liquids. Similarly, the choice of material for constructing the analytical apparatus, that is to say particularly the properties of the walls of the channel system of the inventive apparatus for sample delivery, is subject to no restriction. Pressure variations, pulsations, startup or shut-down effects during sample introduction have no effect on metering accuracy.

The inventive apparatus has broad system-related limits with respect to the delivery volume. The volume of sample liquid which can be injected is determined solely by the volume of the channel section which is situated between the openings. By varying the geometric dimensions of this section in the design of the channel system of the continuous-flow unit, sample volumes matched to the analytical problem can be established in advance. Similarly, it is possible to implement differently sized sections in parallel and/or in series, so that the volume of the section to be displaced by the sample solution can be varied. Preferably, therefore, a system for using the inventive

apparatus is provided with a plurality of channel sections of different dimensions which can be used for sample delivery via respectively independent fluidic connections. As a result, sample volumes between 0.1 and 20 µl at differing levels, according to requirements, can be injected. In this case, usually coefficients of variation during delivery of sample volumes from 1 µl of about 5%, typically less than 2%, are achieved.

In this manner quantitatively reproducible and readily handleable representative sample volumes of a liquid analyte can be introduced into a microstructured system.

Figure shows by way of example a possible arrangement of the channel system of the inventive delivery apparatus. The channel system is subdivided into two channel sections 1A and 1B of differing volumes. Adjacent thereto is the separation channel 1C. Via the fluidic connections 11, 12 and 13, either channel section 1A (when connections 11 and 12 are open) or channel section 1B (during filling via connections 12 and 13) or the two channel sections together (during filling via connections 11 and 13) can be filled with the sample solution. After charging the delivery sections, by applying a voltage the sample in section 1C is separated. If only section 1A was filled with the sample, section 1B can also be used as a separation section, so that the separation section can be lengthened as required.

Figure 2 shows a possible procedure during filling of a miniaturized analytical unit. A channel system is shown which consists of three reservoirs R1 to R3, the channel sections K1 to K4, the fluidic connections F1 to F6 and a branch point Vz. The system shown in the figure has a channel section K1 for sample delivery. Separation can be performed along channel section K2

and K3, or K2 and K4. To carry out an isotachophoretic separation, the system must be charged with a sample and corresponding buffers. In this case the sample volume must be in contact at one end in the direction of the separation section with a buffer (leading buffer) and at the other ēnd with another buffer (terminating buffer). The branching Vz of the channel system gives the possibility of charging differing leading buffers via reservoirs R2 and R3. Fractionated components from the sample can be discharged via the fluidic connection F3.

To achieve the desired arrangement of sample and buffers in the channel system, firstly, as shown diagrammatically under A in the figure, the fluidic connections F2 (outlet), F4, F5 and F6 (inlets) are opened and the channel system is filled via the three reservoirs with the two leading buffers (via R2 and R3, shown with diagonal lines or dots) and the terminating buffer (via R1, shown with vertical stripes). Excess buffer can exit via the fluidic connection F2. In this manner channel section K1 fills with terminating buffer, section K3 with leading buffer (LE2) via R2, section K4 with leading buffer (LE1) via R3 and channel section K2 contains a mixture of the two leading buffers. The fluidic connections F1 and F3 remain closed during this step.

Channel section K2 can optionally be filled with leading buffers via R2 or R3. K2 represents the first section of the separation section.

Part B of the figure shows how the sample is introduced into the channel section K1 and channel section K2 is filled via R3 with a leading buffer. The fluidic connections F5 and F6 are closed and no further terminating buffer is pumped via R1 and no further leading buffer (LE2) via R2. Fluidic connection F4 is open and channel section K2 is filled with leading

buffer (LE1) via R3. At the same time, the fluidic connection F1 is open and the sample is fed via F1 (shown as wavy lines). Excess sample and excess leading buffer (LE1) can exit via the open fluidic connection F2. By pumping the leading buffer (LE1) and the sample volume simultaneously against one another, a particularly precise filling of channel sections K1 and K2 is achieved. In this manner it is also possible to perform an exact charging using pumps which have low pulsation.

After completion of the filling operation, the fluidic connections are closed. In this manner a closed system is obtained without hydrodynamic flow, in which the separation can be carried out reproducibly. The sample can be separated entirely or in fractions via the channel sections K2 and K3 or via the channel sections K2 and K4. As soon as the sample or a selected fraction has migrated through the channel section K2 and arrived at the branch Vz, a decision can be made as to whether separation is to be continued in the direction of K4 or passed to K3. This is achieved by long-term or temporary switching over of the anode potential from F4 to F6.

The table below again shows in outline the fluidic connection circuits during the individual steps of sample delivery:

Filling process	**Fluidic connections**					
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
Filling process A	shut	open "overflow"	shut	open (LE1 in)	open (TE in)	open (LE2 in)
Filling process B	open (in sample)	open, "overflow"	shut	open (LE1 in)	shut	shut

After completion of the filling operation the fluidic connections (F1-F6) are closed.

Below, by way of example, some circuit arrangements for differing analytical processes are listed for an analytical unit corresponding to Figure 2:
(the voltage is applied in each case downstream of the fluidic connections)

1.) Simple separation (separation channels K2 and K4)
anode: F4 cathode: F5

2.) Two-stage separation (discharge into internal channel K3)
a.) separation in K2 anode: F4 cathode: F5
(switched when sample component just before Vz)
b.) separation in K3 anode: F6 cathode: F5

3.) Two-stage separation (dicharge and transfer to external channel)
a.) separation in K2 anode: F4 cathode: F5
(switched when sample component just before Vz)
b.) transfer to exterior via F3
anode: F3 cathode: F5

Figure 3 shows diagrammatically the channel system of a continuous-flow unit having an inventive apparatus for sample delivery and various possibilities for sample removal. The sections and segments of the channel system are labelled K1 to K7, the fluidic connections are labelled F1 to F8 and the detector electrodes D1. The system shown offers the possibility of purifying analytes by
1. the analyte not being moved and the matrix being removed
2. the analyte being removed.

The function of the individual fluidic connections in these two possible procedures is explained below:

F1: Fluidic connection for filling K1 (and any other subsequent channel segments) with final buffer. At F1 there is connection to an external power electrode.

F2: Fluidic connection for charging with primary sample (inlet)

F3: Fluidic connection for displacing the prepared sample from K3 (inlet)

F4: Fluidic connection for removing the prepared primary sample from K3 (outlet)

F5: Fluidic connection for charging the continuous-flow unit with primary sample (outlet)

F6: Fluidic connection for removing the prepared sample from K6 (inlet)

F7: Fluidic connection for removing the prepared sample from K6 (outlet)

F8: Fluidic connection for charging the channel system with leading buffer. At F8 the second external power electrode is attached.

Thus, for case 1, the following procedure results: K1 is charged with end buffer, K2, K3 and K4 with the primary sample and K5, K6 and K7 with the leading buffer. During an isotachophoretic separation, matrix constituents then migrate from K2-K4 into the channel segments K5-K7. The analyte remains in K2-K4. Therefore, at the end of the separation, there remains in K1 end buffer, in K2 to K4 the prepared secondary sample containing the analyte and in K5 to K7 the leading buffer and the removed matrix of the primary sample. The secondary sample containing the analyte can then be removed from K3 via F3 (inlet) and F4 (outlet). Preferably, removal is performed under focussing conditions.

In case 2, the removal of the analyte from the sample, the channel system is charged exactly as in case 1, that is to say K1 is charged with end buffer, K2, K3 and K4 with the primary sample and K5, K6 and K7 with

the leading buffer. During the separation, the analyte migrates from channel segment K2-K4 in the direction of K5-K7. By means of the detector D1 it can be established when the analyte has passed the detection position and separation can be completed at the desired time point. Ideally, the separation is ended when the analyte is in channel section K6. In K1 there is then the end buffer, in K2 to K5 end buffer containing matrix constituents, in K6 the analyte and in K7 the leading buffer and matrix constituents. The analyte can be removed from K6 via F6 (inlet) and F7 (outlet). Removal is preferably performed under a focussing field.

Discharge apparatus:

For the inventive discharge apparatus, the continuous-flow unit channel system must have, in addition to regions for sample delivery and a separation channel, at least one X- or Y-branch departing from a separation channel. For the integration of a plurality of discharge apparatuses, further branches can be introduced at any desired points of the channel system.

The electrodes which are required for the discharge apparatus are transport electrodes which are situated at the ends of the branched channels and permit the potential to be switched over between the two channels, and detection electrodes which are preferably positioned between 40 mm and 0.1 µm, preferably between 20 mm and 0.1 mm, upstream of the branch.

In addition, to control the discharge operation, a switching apparatus is required which can switch over the potential. Switching over can be performed manually or preferably under computer control. In a particularly preferred embodiment, the switching apparatus receives a signal as soon as the analyte has passed the detector

and then automatically switches over the potential at a suitable time point.

Figure 4 illustrates the discharge of a substance using the inventive discharge apparatus. Three different stages of discharge are shown in Figures A, B and C. The diagrammatical discharge apparatus consists of a Y-branched channel system having the transport electrodes 1, 2 and 3 at the ends of the channels. The channel piece between electrodes 1 and 2 serves as separation channel, and the channel branching off to electrode 3 is the discharge channel. Just before branching off of the discharge channel, there is situated in the separation channel a detector electrode 4. In Figure A the substances 5 and 6 to be separated migrate owing to a potential between the electrodes 1 and 2 along the separation channel. Figure B shows the moment when the desired substance 5 passes the detector electrode. The signal detected, for example the specific relative conductivity, causes the potential to be switched over, so that there is then a potential between electrodes 1 and 3. As a result, as shown in Figure C, substance 5 migrates into the discharge channel and is thus separated from substance 6 which is situated in the separation channel. After substance 5 has passed the detector region and has migrated into the discharge channel, the potential can again be switched over so that no further substances pass into the discharge channel.

Fluidic supply:

According to the invention, all valves, pumps or micropumps, tightly closing micropumps, micromixers or other connections of the inventive apparatus which serve for charging the channel system or for removing gas and liquid residues are termed fluidic connections. Generally, according to the invention the fluidic connections are not integrated into the continuous-flow

unit, but are connected to the continuous-flow unit for use externally, that is to say from the adaptor chamber. In this manner, in the continuous-flow unit, only appropriate openings need to be provided, which, in particular for continuous-flow units which are replaced after use, is considerably more inexpensive than the inclusion of expensive valves etc.

Two essential requirements must be made of the hydrodynamic reagent feed and primary sample feed:

1. At the start of the electrophoretic separation, within the continuous-flow unit channel system, the zones of the buffer and of the sample liquid must be reproducibly situated at the positions which are predetermined by the geometry of the continuous-flow unit.
2. During the entire electrophoretic separation, hydrodynamic effects, such as shifting of the liquid column by electoosmosis, must be excluded.

The hydrodynamics can be generated in the inventive analytical unit using commercially available micropumps. However, it has been found that these micropumps frequently have disadvantages, such as inadequate service lives, irreproducible flow rates or gas development under pump load. Therefore, the use of micropumps is not preferred according to the invention. Rather, it has been found that conventional pumps/syringes and valves can be used for the miniaturized application. A precondition for this is synchronization in time of the hydrodynamic processes.

For point 1 it is necessary to remove any gas bubbles present from the channel system. This is readily possible using an active hydrodynamic system, for example a peristaltic pump, by using a gas bolus for collecting smaller gas bubbles. Owing to the volumes which are predetermined by the geometry of the continuous-flow unit and mixing effects which are

negligible in the region of the miniaturized dimensions, it is not necessary to perform precise volumetric metering. The pulsing of the liquid column which accompanies the operation of peristaltic pumps has been found not to be harmful provided that two pumps are in used in synchrony in countercurrent to one another. Similarly, the measurement principle is largely insensitive to variations in flow rates. This permits the use of robust and inexpensive apparatuses, for example peristaltic pumps, syringes or syringe pumps. The use of expensive and fault-susceptible so-called micropumps is not necessary.

For point 2 it is necessary that the continuous-flow unit channel system is hydrodynamically closed during electrophoresis. This is achieved either by pumps having an inherent valve function or by combining pumps with additional valves. The dead volume of the valves used has surprisingly been found not to be harmful provided that closing the valves was carried out synchronized in time.

In the event of this optimized control of hydrodynamic functions, typical volume deviations of less 2% can be achieved.

Power supply:

The power supply serves for carrying out the electrophoretic separation. It is implemented by connecting power electrodes to the continuous-flow unit or, preferably, by contacting power electrodes integrated in the continuous-flow unit via corresponding connections. Preferably, the apparatus for power supply delivers currents between 0 and 50 μA at a maximum voltage of 8 kV. The fluctuation in voltage should not be greater than ± 2%.

Detectors:

The analytes are preferably detected optically or electrochemically. Generally, the detectors of the inventive analytical unit are arranged such that appropriate contact points are situated on the continuous-flow unit which can then be connected externally, that is to say generally from the adaptor chamber. In the event of electric detection, therefore, in the continuous-flow unit there are situated either integrated electrodes which can be contacted externally, or recesses into which electrodes can be reversibly introduced from the outside. The same applies to optical detectors.

In the conductivity measurements, the detector electrodes and the conductivity detector must be decoupled. This is preferably achieved via PTFE (polytetrafluoroethylene)-insulated spools.

For analytical units in which substances are separated electrophoretically, the requirement for a universal detection method is met particularly well by electrical detection methods, such as conductivity measurement. The analytes are characterized in this case via their specific electrical conductivity. A defined substance always generates in a given electrolyte system the same relative conductivity. This applies to sequential measurements in a miniaturized analytical unit and also for measurements which are carried out in a plurality of miniaturized analytical units of a single type.

Preferably, in the inventive apparatus, therefore an electrical conductivity measurement is used which, in the case of directly contacting electrodes, records the electrical current or the electrical voltage drop or,

in the case of galvanically decoupled electrodes, is performed via measurement of the dielectric resistance.

For input or output of optical power into or out of a channel, predominantly methods are used in which optical fibres are positioned directly in front of a glass capillary (for example "classical CE"). For laser-induced fluorescence measurement (LIF) in microstructured channels in planar two-dimensional systems, methods have been established in which the excitation laser light is focused on the channel via open-beam optics and the fluorescence is detected via an open-beam optical system (microscope, possibly confocal, equipped with optical detector, for example CCD camera).

The input and/or output of optical power into defined regions of microstructured planar fluidic systems is achieved in a suitable manner by the arrangement shown in Figure 5. This preferred arrangement permits one optical fibre or a plurality of optical fibres to be led up to the microstructured channels. The arrangement consists of what is termed a double cone (7) into which optical fibres (8) are introduced. This double cone, in this embodiment, seals the channel, that is to say, the fluidic system (6), liquid-tightly and gas-tightly and at the same time makes it possible for input or output of optical power at defined positions in the channel. Since, for the fluidic connection method, essentially the optical fibres are replaced by a tube having very small diameter, fluidic connection and optical connection can be combined in a simple manner. The connection by means of a double cone can therefore similarly be used for fluidic connections, if instead of the optical fibres a tube or capillary is introduced.

In the case of an optical connection, it is also possible for the input of excitation light and output

of emission via the same connection. For this purpose either two fibres are inserted into the cone in order to be able to conduct excitation and emission in two separate fibres, or the excitation wavelength and the emission wavelength are conducted in one fibre. Then, an additional 3rd dB coupler and an optical filter are necessary to eliminate the excitation wavelength.

If the optical fibres have no direct contact with the liquid, in order to avoid fouling of the same, a thin material layer (9) can remain in the substrate (2). The cone in this case has no sealing function.

Adaptor chamber:

The adaptor chamber typically has an apparatus for holding the continuous-flow unit. In addition, it serves for the reversible connection of fluidics, electrics, electronics and optical connections. In this manner, the continuous-flow unit can contain as far as possible only the channel system and necessary recesses for connecting fluidics, electrics etc. All further functionalities are provided by the adaptor chamber and can, if required, be connected to the continuous-flow unit. Thus the continuous-flow unit can be replaced as frequently as desired or can be changed with respect to the design of the channel structure and optional other functions, such as specific detectors, reaction chambers etc. can be changed without the adaptor chamber having to be changed greatly. The adaptor chamber thus contains, for example, a selection of the following functionalities: reversible connections of the power electrodes with the power supply, reversible connection of the detection electrodes with the measuring instrument for electrical conductivity, inlet and outlet capillaries for separation buffer and sample material, connections for invasive detectors (potentiometric or amperometric detectors, fibre-optic guides for measurement of transmitted light, scattered

light or fluorescence etc.), outgoing capillaries for discharging separated-off components, cooling apparatus for removing Joule heat during electrophoresis, device for monitoring atmospheric humidity and dust particle density in the surroundings of the continuous-flow unit.

Functionalities are typically connected to the continuous-flow unit via a holder integrated into the adaptor chamber, generally in the form of a plate. On this holder are situated, at positions which correspond to appropriate recesses in the continuous-flow unit, matching connection elements. According to the invention connection elements are connections which ensure connection between the continuous-flow unit and the functionalities in the adaptor chamber. The force which is necessary for sealing between the connection elements and the openings on the continuous-flow unit is preferably provided via a press-on plate which presses the holder containing the connection elements onto the continuous-flow unit. The connection elements are preferably supplied from the rear side of the holder via feed lines.

In another preferred embodiment, the connection elements are not fixed in precise positions in a holder, but can be connected via variable tubings or telescope arms to any desired position in the continuous-flow unit. In this case, each connection must be sealed individually, for example, via clamps etc. This embodiment permits a greater variability with respect to the continuous-flow unit design, but requires greater effort during its connection.

In a particularly preferred embodiment, therefore, the power electrodes and detection electrodes are connected to the power supply and the conductivity detector via telescopic electrodes which are mounted on one side of the holder. The fluidic connections, in contrast, are

mounted on the holder in precise positions corresponding to the continuous-flow unit. If a continuous-flow unit with altered capillary geometry is to be used, the holder must be exchanged for a holder having correspondingly positioned fluidic connections.

Figure 6 shows diagrammatically the connection of fluidics and electrics to a continuous-flow unit. The continuous-flow unit consists of a substrate (1) and a cover (2). The substrate (1) is microstructured, so that the channel system (3) is formed. On the cover (2) is applied a power electrode or detection electrode (4). The continuous-flow unit is held by a holding apparatus (5). Above the continuous-flow unit is the holder (6) containing the connection elements, a fluidics connection (8a-8c) and an electrode connection (9a-9c). The fluidics connection is here additionally held via an exchangeable sealing plate (7) having contact elements. The fluidics connection essentially consists of a tube connection (8a) having a press-on screw for fastening and sealing the feeding capillary, a sealing element (8b) and a further sealing element (8c) which can be introduced as an exact fit into the recess in the substrate (1) and thus effects the connection between the fluidic connection and the channel structure. The electrode connection essentially consists of an electrical contact for high voltage or for detection (9a), a spring (9b) and a contact part (9c) which can be brought into contact with the electrode (4) by the spring.

The inventive analytical unit, including the power supply, the switching over of the power, the detectors, the fluidic connections, is preferably controlled by means of appropriate computer systems. It is also possible to provide manual control for certain switching operations. If the analytical unit has an apparatus for discharging substances, typically, the

switching arrangement necessary for this operation is also integrated into the general switching system.

The inventive analytical unit, by the combination of the inventive sample delivery, the possibility of integrating electrodes at any desired position of the continuous-flow unit and the inventive discharge apparatus, makes it possible to carry out the most varied types of separations and analyses. Since very high sample volumes can be delivered, the analytical unit is suitable, in particular, for sample preparation. For example, the following separation problems and analytical problems can be dealt with:

1. Depletion of matrix components from a primary sample, preferably if the primary sample has a significant content of ionic matrix components which have a higher electrophoretic mobility than the analyte.

For example, these are analytes, such as proteins, complex carbohydrates or fragments of biological membranes. Low-molecular-weight salts are electrophoretically extracted from the primary sample. The secondary sample thus produced is then preferably hydrodynamically fed to a further analytical process. More details on matrix depletion in the case of acidic or basic proteins may be found in Examples 1 and 2.

2. Extraction of analytes from the primary sample, preferably when the primary sample has a significant content of matrix components which have a lower electrophoretic mobility than the analyte.

One example of this is the removal of alkali metal cations from serum, plasma or whole blood (see Example 3). In this example, it must be ensured that dilution of the extracted electrolytes does not occur in the secondary sample. The continuous-flow unit,

because of the matrix residues remaining after the extraction must either be exchanged or purified.

3. Removal of the analytes from the primary sample when not only significant contents of electrophoretically more mobile constituents but also electrophoretically less mobile constituents are present.
This case which is frequent in practice, demands monitoring of the electrophoretic separation, in which case the volume elements which contain analytes 1. must be identified, 2. their geometric extent must be determined and 3. the volume elements of the secondary sample must be removed from the volume elements of the matrix by controlled fluidic operation (discharge). Example 4 shows the separation of organic acids from wine.

4. Enrichments of analytes present in minor amounts
The inventive analytical unit permits the enrichment of analytes which are present in traces in a matrix of substances having similar electrophoretic mobility, if conditions can be found under which the electrokinetic mobilities of the analytes differ by a few percent from the elecrokinetic mobilities of the excess components. The method of isotachophoresis makes it possible to separate dissolved ionic components. In contrast to chromatographic methods, molecular interactions between components of the primary sample with a stationary heterogeneous phase do not play a role. Similarly, no homogeneous stationary phases such as immobilized buffers or pore-forming gels are used. In addition, in the course of the separation of the analytes, there is no increasing dilution with the solvent. In contrast, it is possible to concentrate the analytes in homogeneous zones.

In addition to the separation and concentration of the trace analytes, it must be ensured that the very small volume element which contains the trace analytes can be

detected in a simple manner. For this, preferably, what is termed a spacer is used which is added to the primary sample. The spacer has a similar electrophoretic mobility to the analyte and is detected and selected together with it. The spacer has no effect or only a slight effect on the complete analytical process with the secondary sample. In examples 4 b) and c) in each case aspartate was added to the sample as internal standard and spacer between gluconate and succinate.

Preferably, the inventive analytical unit is used in such a manner that series of sample feeds following each other in time can be carried out without replacing reagents or the continuous-flow unit. After completion of the series, the reagents and the continuous-flow unit can be replaced in a simple manner. A great advantage of the invention is that the analytical performance of the sample preparation is repeatedly available over a long period without maintenance expenditure and the place and time point of the analytical use can be selected in a broad range. In a preferred embodiment, the inventive analytical unit combines the advantages of a software-monitored complete system: standardizability of sample preparation, repeatability of separation, quality control, intrinsic error detection, with the advantages of miniaturization, such as low instrument costs, mobility, small size, low operating costs and simple operation.

In addition, it is possible in a simple manner to bring various detectors into direct contact with the fractionated sample material and to use the apparatus in a simple manner for sample preparation and as a miniaturized analytical system.

Even without further details, it is assumed that those skilled in the art can use the above description to the

broadest extent. The preferred embodiments and examples are therefore to be interpreted as descriptive, but in no way as a disclosure limiting in any manner.

The complete disclosure of all applications, patents and publications mentioned - above and below, in particular of the corresponding applications DE 199 27 533.5, DE 199 27 534.3 and DE 199 27 535.1 submitted on 16.06.1999, and PCT/EP 00/05204, PCT/EP 00/05205 and PCT/EP 00/05206, submitted on 06.06.2000, is incorporated by reference into this application.

Examples

1st example Matrix depletion in the case of acidic proteins

The primary sample contains acidic proteins having isoionic points in the range from pI=5 to pI=6. Other matrix constituents, but particularly basic proteins having isoionic points in the range pI=9 to pI=10 are to be separated off from this protein fraction.
Separation conditions:
The power electrode of the terminating buffer is connected as cathode. The pH of the leading buffer is in the region of pH = 5.5. Under these conditions, the acidic proteins have a negligible net charge and do not migrate in the electric field, but remain during the isotachophoresis in the sample application segment. The basic proteins, in contrast, have a positive surplus charge and migrate towards the cathode. Depending on the objective, either only the matrix-depleted acidic proteins can be taken off from the sample application segment as secondary sample, or, in addition, the basic proteins can be taken off from a separation channel segment. Isolation of the basic proteins requires a longer separation time.
Electrolyte system:

Leading buffer: 20 mM sodium acetate + acetic acid + MHEC
(methyl hydroxyethyl cellulose for suppressing the electroosmotic flow) pH 5.5
Terminating buffer: 10 mM acetic acid

2nd example Matrix depletion in the case of basic proteins

The sample composition corresponds to Example 1.
The power electrode of the terminating buffer is connected as anode. The pH of the leading buffer is in the range from pH = 9 to pH = 10, so that proteins having isoionic points from 9 to 10 do not migrate in the electrophoretic field..
Buffer system:
Leading buffer: 20 mM HCl or acetic acid, MHEC, pH = 9.5.
Terminating buffer: 20 mM bis-tris propane, pH = 10.5.

Other buffer systems which are suitable for electrophoretic matrix separation from proteins according to their isoionic points are listed below:
The power electrode of the leading buffer is connected as anode in each case:

pH 6-7
Leading buffer: 20 mM HCl + histidine + MHEC,
Terminating buffer: 10 mM morpholinomethanesulphonic acid, MES, + histidine.

pH 7-8
Leading buffer: 20 mM HCl + imidazole + MHEC,
Terminating buffer: 10 mM 4-morpholinepropanesulphonic acid + imidazole.

pH 8-9
Leading buffer: 20 mM HCl + trishydroxymethylamino-methane TRIS + MHEC

Terminating buffer: 10 mM N-tris(hydroxymethyl)methyl-3-aminopropanesulphonic acid, TAPS, + TRIS

3rd example Removal of cations from serum

The primary sample, serum, is diluted 10-fold with water and filtered through a 0.45 µm membrane.
Leading buffer: 15 mM caesium acetate + tartaric acid + 75 mM 18-crown-6-ether + 10% polyethylene glycol (300) + 0.1% polyethylene glycol (5 000 000), pH = 5.0
Terminating buffer: 10 mM zinc acetate + acetic acid, pH = 5.8.
Current: 1, 2: 15 mA

The analytical result is shown in Figure 7 (the time in seconds is plotted on the x-axis).
1 = ammonium
2 = sodium
3 = magnesium
4 = calcium
5 = potassium
R = resistance
LE =leading buffer
TE = terminating buffer

4th example Analysis of wine

Separation conditions:

LE:	10 mmol/l HCl + β-alanine + 0.1% methyl hydroxyethyl cellulose, pH = 2.9
TE 1:	5 mmol/l caproic acid + histidine, pH = 6.0
TE 2:	5 mmol/l glutamic acid + histidine, pH = 5.0

Figures 8 to 10 show the separation of the following samples. The time in seconds is plotted on the x-axis and the resistance R on the y-axis. The aspartate added in Example 4 b) and c) acts as internal standard and as spacer between gluconate (9) and succinate (11).

a)

<u>Fig. 8:</u>

0.2 mmol/l sulphate, sulphite, phosphate, malonate, tartrate, citrate, malate, lactate, gluconate, aspartate, succinate, acetate, ascorbate, sorbate

Current 1: 10 µA

Current 2: 10 µA

b)

<u>Fig. 9:</u>

20-fold diluted white wine + 0.25 mmol/l aspartate

Current 1: 20 µA

Current 2: 10 µA

c)

<u>Fig. 10:</u>

20-fold diluted red wine + 0.25 mmol/l aspartate

Current 1: 20 µA

Current 2: 10 µA

The numbering in Figures 8 to 10 indicates the following constituents:

1 = sulphate
2 = sulphite
3 = phosphate
4 = malonate
5 = tartrate
6 = citrate
7 = malate
8 = lactate
9 = gluconate
10 = aspartate as internal standard
11 = succinate
12 = ascorbate
13 = acetate
14 = sorbate
i = impurities

Claims

1. Analytical unit for sample preparation at least comprising a continuous-flow unit made of plastic having a microstructured channel system, an adaptor chamber for reversibly receiving the continuous-flow unit, a fluidics supply, a power supply and at least one detector, characterized in that to receive the sample a channel section is provided at the ends of which in each case are situated fluidic connections.

2. Analytical unit according to Claim 1, characterized in that integrated into the analytical unit is an apparatus for discharging sample volumes, which apparatus essentially consists of a channel system having at least one Y branch, at least three transport electrodes and at least one detection apparatus upstream of the said branching point of the channel system and an electrical switching apparatus.

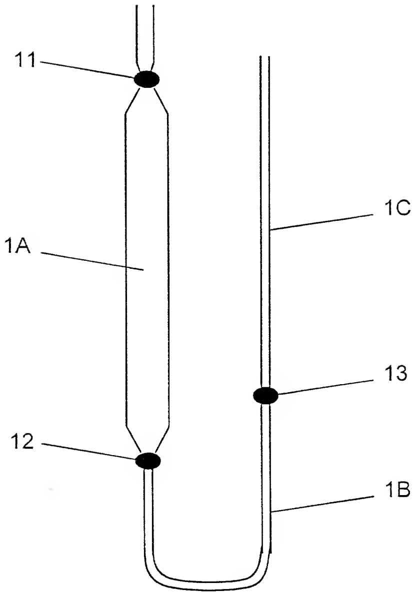
3. Analytical unit according to Claim 1 or 2, characterized in that the fluidic connections are peristaltic pumps, syringes or syringe pumps.

4. Use of the analytical unit according to one of Claims 1 to 3 for isotachophoretic separation of a sample.

5. Use of the analytical unit according to one of Claims 1 to 3 for depleting matrix components from a primary sample, for extracting analytes from a primary sample, for separating off analytes from the primary sample or for enriching of analytes in minor amounts.

10/009635

Fig. 1



10009635 .121701

Fig. 2

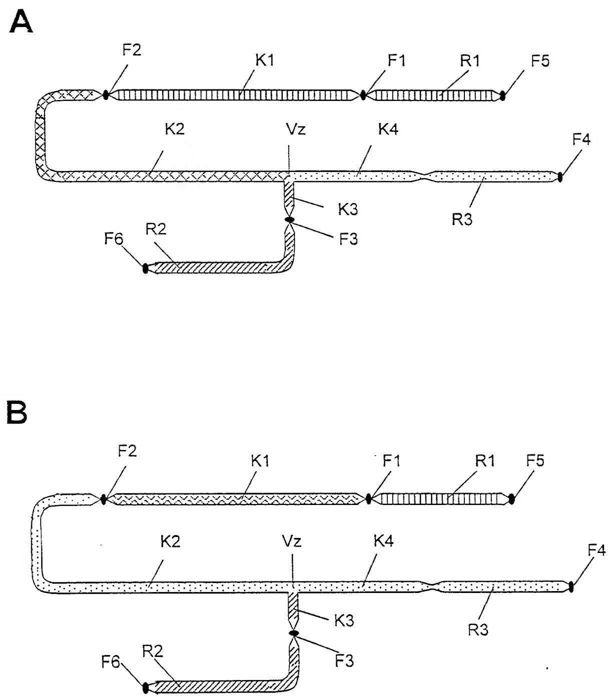

A
F2
K1
F1
R1
F5
K2
Vz
K4
F4
K3
R3
F6
R2
F3
B
F2
K1
F1
R1
F5
K2
Vz
K4
F4
K3
R3
F6
R2
F3

Fig. 3

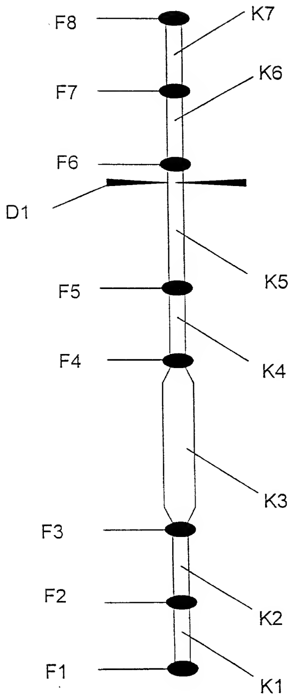


Fig. 4

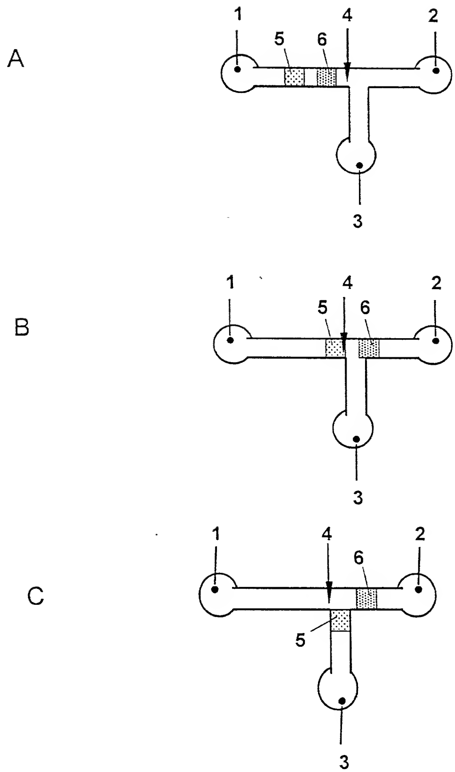


Fig. 5

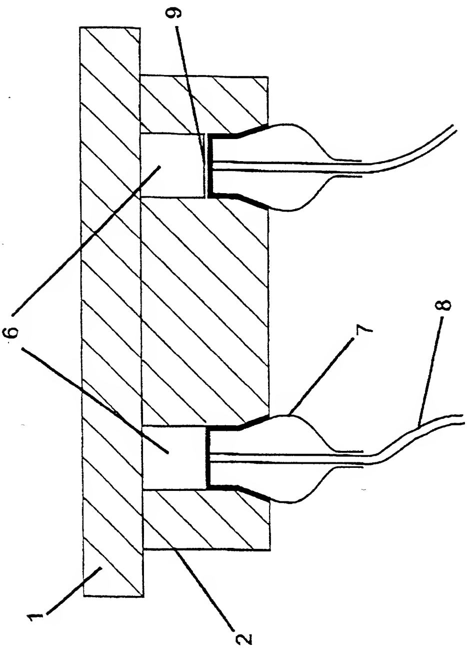
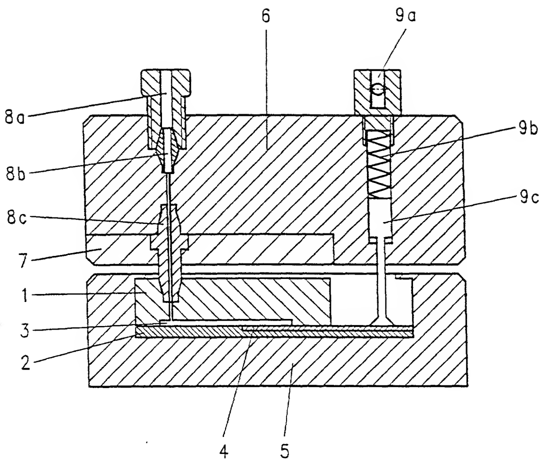
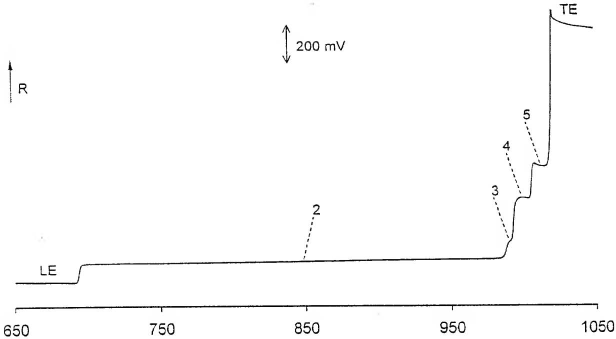


Fig. 6



10005655.140404

Fig. 7



10009635.121001

Fig. 8

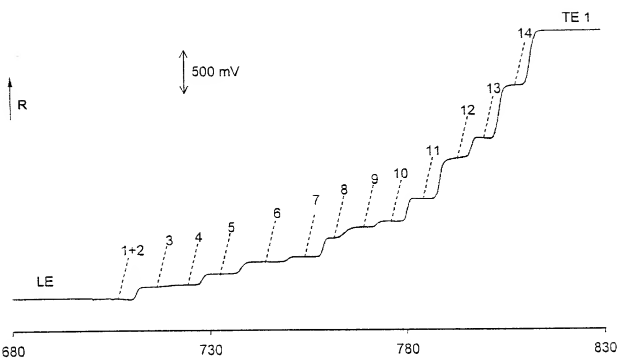


Fig. 9

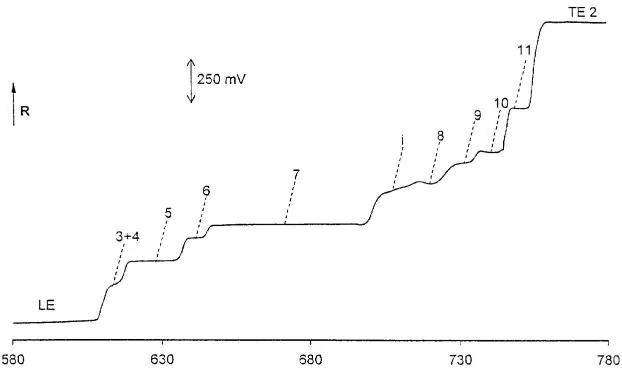
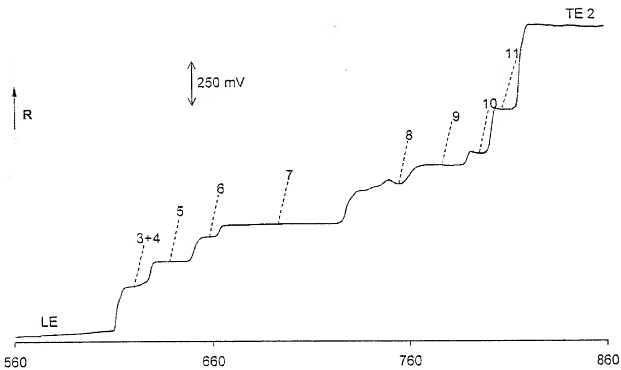


Fig. 10



10/009635 . 121401

Express Mail Label No.

Docket No.
Merck

Declaration and Power of Attorney For Patent Application
English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

Device for preparing samples

the specification of which

(check one)

☐ is attached hereto.
☒ was filed on June 15, 2000 as United States Application No. or PCT International Application Number PCT/EP00/05518
and was amended on ____________
(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Not Claimed
199 27 533.3 (Number)	Germany (Country)	16.06.1999 (Day/Month/Year Filed)	☐
199 27 534.3 (Number)	Germany (Country)	16.06.1999 (Day/Month/Year Filed)	☐
199 27 535.1 (Number)	Germany (Country)	16.06.1999 (Day/Month/Year Filed)	☐
PCT/EP00/05204 (Number)	PCT (Country)	06.06.2000 (Day/Month/Year Filed)	☐
PCT/EP00/05205 (Number)	PCT (Country)	06.06.2000 (Day/Month/Year Filed)	☐
PCT/EP00/05206 (Number)	PCT (Country)	06.06.2000 (Day/Month/Year Filed)	☐

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

(Application Serial No.) (Filing Date)

(Application Serial No.) (Filing Date)

(Application Serial No.) (Filing Date)

I hereby claim the benefit under 35 U.S.C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States of PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112. I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C.F.R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint

I. William Millen (Reg. No. 19,544)
John L. White (Reg. No. 17,746)
Anthony J. Zelano (Reg. No. 27,969)
Alan E.J. Branigan (Reg. No. 20,565)
John R. Moses (Reg. No. 24,983)
Harry B. Shubin (Reg. No. 32,004)
Brion P. Heaney (Reg. No. 32,542)
Richard J. Traverso (Reg. No. 30,595)
John A. Sopp (Reg. No. 33,103)
Richard M. Lebovitz (Reg. No. 37,067)
John H. Thomas (Reg. No. 33,460)
Catherine M. Joyce (Reg. No. 40,668)
James T. Moore (Reg. No. 35,619)
James E. Ruland (Reg. No. 37,432)
Nancy Axelrod (Reg. No. 44,014)
Jennifer J. Branigan (Reg. No. 40,921)

to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

Send Correspondence to: MILLEN; WHITE; ZELANO & BRANIGAN, P.C.
Arlington Courthouse Plaza I
2200 Clarendon Blvd., Suite 1400
Arlington, VA 22201

Direct Telephone Calls to: (name and telephone number)

Full name of sole or first inventor	
Friedhelm Eisenbeiß	
Sole or first inventor's	Date December 7, 2001
Residence Luisenstrasse 28, 64331 Weiterstadt, Germany	
Citizenship German	DEX
Post Office Address c/o Merck KGaA, Frankfurter Str. 250, 64293 Darmstadt, Germany	

Full name of second inventor, if any	
Bernd Stanislawski	
Second inventor's signature	Date December 7, 2001
Residence Ilkenhansstrasse 13, 60433 Frankfurt, Germany	
Citizenship German	DEX
Post Office Address c/o Merck KGaA, Frankfurter Str. 250, 64293 Darmstadt, Germany	

3-00

Full name of third inventor, if any

Thomas Greve

Third inventor's signature | Date

Thomas Greve | December 7, 2001

Residence

Dieburger Strasse 238, 64287 Darmstadt, Germany

Citizenship

German DEX

Post Office Address

c/o Merck KGaA, Frankfurter Str. 250, 64293 Darmstadt, Germany

4-00

Full name of fourth inventor, if any

Renate Bender

Fourth inventor's signature | Date

Renate Bender | December 7, 2001

Residence

Altheimweg 14, 64291 Darmstadt, Germany

Citizenship

German DEX

Post Office Address

c/o Merck KGaA, Frankfurter Str. 250, 64293 Darmstadt, Germany

5-00

Full name of fifth inventor, if any

Roland Hergenröder

Fifth inventor's signature | Date

December 7, 2001

Residence

Immermannstrasse 35, 44147 Dortmund, Germany

Citizenship

German DEX

Post Office Address

c/o Merck KGaA, Frankfurter Str. 250, 64293 Darmstadt, Germany

6-00

Full name of sixth inventor, if any

Günther Weber

Sixth inventor's signature | Date

December 7, 2001

Residence

Justusweg 2, 44149 Dortmund, Germany

Citizenship

German

Post Office Address

c/o Merck KGaA, Frankfurter Str. 250, 64293 Darmstadt, Germany

7-00

Full name of third inventor, if any	
Benedikt Graß	
Third inventor's signature	Date
Benedikt Graß	December 7, 2001
Residence	
Schlesien Strasse 30, 69457 Werl, Germany	
Citizenship	
German	DEX
Post Office Address	
c/o Merck KGaA, Frankfurter Str. 250, 64293 Darmstadt, Germany	

8-00

Full name of fourth inventor, if any	
Andreas Neyer	
Fourth inventor's signature	Date
Andreas Neyer	December 7, 2001
Residence	
Langerfelderstrasse 69a, 58838 Iserlohn, Germany	
Citizenship	
German	DEX
Post Office Address	
c/o Merck KGaA, Frankfurter Str. 250, 64293 Darmstadt, Germany	

9-00

Full name of fifth inventor, if any	
Matthias Jöhnck	
Fifth inventor's signature	Date
Matthias Jöhnck	December 7, 2001
Residence	
Dülmener Strasse 27a, 48163 Münster, Germany	
Citizenship	
German	DEX
Post Office Address	
c/o Merck KGaA, Frankfurter Str. 250, 64293 Darmstadt, Germany	

10-00

Full name of sixth inventor, if any	
Dirk Siepe	
Sixth inventor's signature	Date
Dirk Siepe	December 7, 2001
Residence	
Vinckeplatz 7, 44139 Dortmund, Germany	
Citizenship	
German	DEX
Post Office Address	
c/o Merck KGaA, Frankfurter Str. 250, 64293 Darmstadt, Germany	

11-00

Full name of third inventor, if any	
Dusan Kaniansky	
Third inventor's signature D. Kaniansky	Date December 7, 2001
Residence Sevcenkova 28, 85101 Bratislava. Slovakia	
Citizenship SK	DaK
Post Office Address c/o Merck KGaA, Frankfurter Str. 250, 64293 Darmstadt, Germany	

Full name of fourth inventor, if any	
Fourth inventor's signature	Date
Residence	
Citizenship	
Post Office Address	

Full name of fifth inventor, if any	
Fifth inventor's signature	Date
Residence	
Citizenship	
Post Office Address	

Full name of sixth inventor, if any	
Sixth inventor's signature	Date
Residence	
Citizenship	
Post Office Address	